Loss of Chromosome 18 in Neuroendocrine Tumors of the Small Intestine: The Enigma Remains

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Abstract

Background/Aims: Neuroendocrine tumors of the small intestine (SI-NETs) exhibit an increasing incidence and high mortality rate. Until now, no fundamental molecular event has been linked to the tumorigenesis and progression of these tumors. Only the loss of chromosome 18 (Chr18) has been shown in up to two thirds of SI-NETs, whereby the significance of this alteration is still not understood. We therefore performed the first comprehensive study to identify Chr18-related events at the genetic, epigenetic and gene/protein expression levels. Methods: We did expression analysis of all seven putative Chr18-related tumor suppressors by quantitative real-time PCR (qRT-PCR), Western blot and immunohistochemistry. Next-generation exome sequencing and SNP array analysis were performed with five SI-NETs with (partial) loss of Chr18. Finally, we analyzed all microRNAs (miRNAs) located on Chr18 by qRT-PCR, comparing Chr18+/– and Chr18+/+ SI-NETs. Results: Only DCC (deleted in colorectal cancer) revealed loss of/greatly reduced expression in 6/21 cases (29%). No relevant loss of SMAD2, SMAD4, elongin A3 and CABLES was detected. PMAIP1 and maspin were absent at the protein level. Next-generation sequencing did not reveal relevant recurrent somatic mutations on Chr18 either in an exploratory cohort of five SI-NETs, or in a valida-
Introduction

Neuroendocrine tumors (NETs) of the gastrointestinal tract are considered to be rare tumors, representing only 2% of all gastrointestinal neoplasms. However, NETs of the small intestine (SI-NETs) are the most common tumors of this part of the gastrointestinal system [1] and have experienced a dramatic increase in incidence over the past three decades [2]. SI-NETs are tumors with low rates of somatic mutations, as shown in 2 recent studies involving the analysis of 48 and 180 SI-NETs, respectively, by massively parallel sequencing, which revealed a mutation frequency (single nucleotide variants; SNVs) of approximately 0.1 per 10^6 nucleotides. PI3K/Akt/mTOR signaling molecules showed copy number changes in 29% of the analyzed cases. Hedgehogygous frameshift mutations of *CDKN1B*, encoding the tumor suppressor p27, were found in 8% of the cases, while monoallelic deletions of *SMAD2* and *SMAD4* [3, 4]. *SMAD2* and *SMAD4* are located in close proximity to each other on chromosome 18 (Chr18), whose loss is the most common event in SI-NETs [5], but the role of this frequent genetic aberration for the tumor progression of SI-NETs has not yet been clarified. The loss of one Chr18 allele could possibly result in the partial loss of tumor suppressor proteins located on Chr18, such as *SMAD2*, *SMAD4*, DCC (deleted in colorectal cancer) and maspin. A recent integrated (epi)genomic and transcriptomic study identified 25 Chr18-related genes to be differentially methylated between tumors affected by Chr18 loss of heterozygosity (LOH) and normal small intestine. For some of these genes, the aberrant methylation pattern was associated with reduced mRNA expression, e.g. *SERPINB5*, coding for maspin. In addition, the authors were able to classify SI-NETs in three distinct subgroups, with the Chr18 LOH group being the most favorable and significantly associated with reduced global methylation status [in contrast to no copy number variations (CNVs) and multiple CNVs] [6]. Although these recent reports gave important new insights into the genetic events in SI-NET, not one of the putative tumor suppressors or oncogenic proteins has been studied at the protein level.

Other proteins that have been discussed are *PMAIP1* and CABLES [7, 8]. Single point mutations or deletions/insertions in genes, especially tumor suppressors of Chr18 regions that show an LOH could result in a total loss of function. Imprinted genes requiring only one mutational hit for inactivation are of special interest regarding potential tumor suppressor genes. *TCEB3C*, an imprinted gene located on Chr18, functions as a transcription elongation factor and, hence, is an interesting tumor suppressor candidate [9]. Furthermore, epigenetic modifications such as promoter methylation of Chr18 genes and/or LOHs may have an impact on the tumorigenesis of SI-NETs.

In addition to the loss of protein expression of tumor suppressors, loss of Chr18 may result in a downregulation of Chr18-related microRNAs (miRNAs) involved in tumor progression. Similar effects on miRNA expression by chromosome loss have been described for gastrointestinal stromal tumors in which the partial loss of chromosome 14 went hand in hand with the downregulation of 38 chromosome 14-related miRNAs [10]. Another, albeit unlikely, possibility is that the loss of Chr18 represents a passenger gene alteration with no distinct impact on the tumor progression.

In order to address these questions, we performed the first systematic study on Chr18-related alterations at the protein level in SI-NETs, complemented with the assessment of genetic alterations such as SNVs, insertions/deletions (indels), gain and loss as well as LOH. Our experimental setup is depicted in a flow chart which can be found in the online supplementary data 1 (see www.karger.com/doi/10.1159/000446917 for all online suppl. material). Online supplementary table S1 summarizes all analyses we performed (in detail and in a compact overview) and the samples which were used.

Material and Methods

Material and methods can be found in the online supplementary data 2.

Results

Chr18 Is Commonly Lost in SI-NETs

Focusing on this characteristic lesion, FISH analysis with a Chr18 centromeric enumeration probe was performed on 132 SI-NET samples. The analysis was possible...
in 95% (125/132) of all cases. It revealed that 71% of the tumor samples (89/125) had a loss of Chr18. 11% showed mosaicism regarding their Chr18 status (14/125); 18% exhibited the normal count of two copies of Chr18 (22/125). In 5% (7/132), it was not possible to determine the Chr18 status due to tissue degradation. In the three cohorts, 57% of cohort 1, 65% of cohort 2 and 65% of cohort 3 showed a loss of Chr18.

The Chr18 status for the 5 samples used for exome sequencing (Tu1 Met, Tu3, Tu4, Tu5 Met and Tu7 Met) and one additional sample for SMAD2/4 Western blot (Tu6 Met) was evaluated by SNP array and revealed 4 samples to have lost one copy of Chr18, whereas one sample depicted mosaicism (Tu3) and one showed the normal count of two copies (Tu6 Met). The clinical data of all cases can be found in online supplementary table S2.

Expression of Chr18-Related Tumor Suppressor Genes in SI-NETs

The common loss of Chr18 in SI-NETs suggests that tumor suppressor genes associated with Chr18 play a role in the tumorigenesis of this rare type of tumors.

SMAD2 and SMAD4 Are Strongly Expressed in SI-NETs

In order to analyze whether Chr18 loss is accompanied with a loss of protein expression of the two tumor suppressors SMAD2 and/or SMAD4, Western blot analyses were performed with protein lysates from 14 SI-NET samples (10 with loss of Chr18, confirmed by Chr18 centromere FISH, 2 without loss of Chr18 and 2 samples with a mosaicism regarding Chr18 status). Both potential tumor suppressor proteins were detected in varying amounts in all samples investigated (fig. 1, online suppl. table S3A). Nevertheless, we found that the SI-NET samples showed differences in the strength of expression of SMAD2, whereas the β-actin expression varied only slightly between samples. One sample was negative for SMAD2 (sample 6, <20% of β-actin expression). This sample depicted loss of Chr18. Half of the samples showed (slightly) higher SMAD4 expression than expression of the housekeeping protein β-actin, whereas the other half of the samples showed similar expression for both proteins.

A total of 84 out of 87 tumor samples (97%) exhibited strong nuclear expression of SMAD4 in immunohistochemical staining (fig. 2a). Three samples showed no expression of this protein, whereas the corresponding epithelium of the normal mucosa still expressed SMAD4 (fig. 2b). However, compared to the loss of SMAD4 in pancreatic ductal adenocarcinomas, the loss in our SI-NET samples is somehow less prominent (fig. 2b, small picture).

The immunohistochemical staining of SMAD2 revealed no difference of expression in the tumor tissues compared to the corresponding normal tissues (fig. 2c). The protein is strongly expressed in the nuclei of SI-NETs, but to the same degree in the nuclei of the adjacent normal epithelium.

Maspin Is Not Expressed by Neuroendocrine Cells of the Ileal Mucosa

The immunohistochemical staining for maspin was negative in all SI-NET samples; in contrast, the normal mucosa exhibited expression of this protein (fig. 2d). In order to explore whether the lacking expression of maspin represents a loss of expression during tumorigenesis or whether, instead, it belongs to the normal phenotype of neuroendocrine (NE) cells of the small intestine, double immunofluorescence stainings of synaptophysin and maspin were conducted on 20 normal small intestine tissues adjacent to SI-NETs. This experiment revealed that NE cells of the ileal mucosa do not express maspin, in contrast to mucin-producing cells of the intestinal mucosa (fig. 2e).
Elongin A3 and CABLES Show Strong Expression in SI-NETs, whereas PMAIP1 Is Not Expressed

Western blot analyses of the two tumor suppressors elongin A3 and CABLES were performed with 21 fresh frozen tissues. CABLES was detected in varying amounts in all analyzed samples (fig. 3, upper panel, only the first 8 samples are shown). Since two different bands were visible in the elongin A3 Western blot, a competition analysis was performed, identifying the upper band to be the specific one (fig. 3, middle panel; only the first 8 samples are shown). Elongin A3 exhibited distinct positive results in 20 of the 21 samples; one sample was negative (not shown).

Western blot analysis of PMAIP1 revealed negativity (<20% of β-actin expression) of the first 8 samples (fig. 3, lower panel); therefore, no further samples were tested.
Quantitative Western blot results are shown in online supplementary table S3B.

DCC Expression Is Lost/Reduced in Nearly 30% of SI-NET Samples

Western blot analysis of DCC using the first antibody was performed with 21 tumor samples. The evaluation of the Western blot results revealed total loss or reduced DCC expression (<20% of β-actin expression) in 29% (6/21) of samples (fig. 4a, online suppl. table S3C).

With the first 8 tumor samples, a second Western blot was performed, using another antibody (fig. 4b). Due to difficulties in distinguishing the bands between 180 and 250 kDa, a competition with the matching peptide was done. In addition, a label-free identification of DCC-specific peptides in the positive control IMR-32 by mass spectrometry was performed. The mass spectrometry confirmed DCC to be present in the gel bands at ∼190 kDa.

The second Western blot analysis revealed samples 5 and 7 to have lost DCC expression. This result was supported by the first Western blot: sample 7 lost DCC expression as well and sample 5 exhibited reduced expression of DCC. Since the background signal was very high, samples 3 and 6 were not analyzable in the second Western blot. All samples with lost/reduced expression of DCC depicted only one copy of Chr18.

Real-Time PCR Analysis Reveals TCEB3C and CABLES to Be Differentially Expressed between Sample Cohorts with and without Loss of Chr18

Since only one cryo-sample with the normal count of Chr18 was available for Western blot analyses of TCEB3C, PMAIP1, DCC and CABLES, we decided to additionally perform quantitative real-time PCR (qRT-PCR) to address the question of the influence of the Chr18 loss on these tumor suppressors.

The qRT-PCR of TCEB3C, PMAIP1, DCC and CABLES in a great cohort of 69 formalin-fixed paraffin-embedded (FFPE) samples showed no common loss of one of the potential tumor suppressor genes with the Cp values ranging between 22 (high expression) and 35 (low expression).

Four samples had Cp values >30 in the normalization control of β-Actin and were therefore excluded from further analysis. Of the remaining 65 samples, 38 exhibited the loss of one Chr18, 13 samples had the normal count of two Chr18, and 12 tumors showed mosaicism regard-
ing their Chr18 status. It was not possible to ascertain the Chr18 status for 2 samples.

The comparative analysis of cohorts with/without loss of Chr18 (the mosaic samples were excluded from this analysis) revealed a significant downregulation of TCEB3C (p = 0.0101) and CABLES (p = 0.0323) in the cohort with the loss of Chr18 (n = 38) compared to the cohort with the normal Chr18 count (n = 13). No significant difference in expression between the two cohorts was detected for DCC (p = 0.2151 for Ex3–4 and p = 0.3134 for Ex17–18, respectively) and PMAIP1 (p = 0.0652) (fig. 5).

**No Additional LOH Detected**

SNP array analysis of six SI-NETs revealed 4 samples to have a loss of Chr18; one primary tumor exhibited mosaicism regarding Chr18 status and one sample depicted the normal count of two Chr18. In the samples with a loss

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**Fig. 4.** a Western blot of the Chr18-associated tumor suppressor DCC. DCC is lost in samples 7, 12 and 16. Samples 3, 5, 10, 11 and 17 show greatly reduced expression of DCC (158 kDa). b DCC Western blot of the first 8 tumor samples, using a second antibody. A competition analysis with the matching peptide was carried out to determine the right protein band. DCC is lost in samples 5 and 7 (190 kDa). Due to the strong background, samples 3 and 6 are not analyzable. M = Mosaicism; 1 = 1 × Chr18; 2 = 2 × Chr18; P = positive control. Numbers indicate tumor content; red arrows indicate specific bands.
of Chr18, no further losses (with the analysis settings explained in online suppl. data 2: SNP array analysis) on the remaining Chr18 could be detected.

No Recurrent Mutations in Chr18-Associated Genes Detected

Exome sequencing was performed with 5 different SI-NET samples (4 with loss of Chr18, 1 with mosaic Chr18 pattern) and corresponding normal tissue samples. Statistics concerning the mean coverage on target and underrepresented bases (total and in percent) are shown in online supplementary table S4. Three somatic mutations in genes located on Chr18 were identified (see online suppl. table S6). SNVs in **CABYR** and **NFATC1** were found in 1 patient, while a 3-bp deletion in **PIEZO2** was present in another patient. The SNV in **NFATC1** was predicted to be deleterious/damaging (Provean/SIFT). In contrast, the SNV in **CABYR** was predicted to have no damaging effect on protein structure/function (neutral/tolerated). The 3-base pair deletion in **PIEZO2** was predicted to have a deleterious effect on protein structure/function. The remaining 3 samples showed no SNVs or indels in Chr18-related genes.

All 3 mutations were Sanger validated with DNA extracted from fresh frozen tissue (tumor as well as corresponding normal tissue) confirming a somatic origin. **NFATC1** was analyzed in a larger cohort of 30 SI-NETs (15 primaries – 8 with matching metastases – 7 non-matching metastases) in which no genetic alterations were found.

Data Comparison with External Data Set

Banck et al. [4] detected 197 somatic alterations in 48 patients. Concerning Chr18, they identified only one SNV. The gene ANKRD30B exhibited a T > A substitution at position 1476361 (p.V625D, predicted to be benign). The tumor in which the mutation was found showed no loss of Chr18. In comparison, in our exome sequencing of 5 patients with SI-NETs, we found two SNVs and one deletion in genes on Chr18 in 2 patients.
The tumor that had mutations in NFATC1 and CABYR exhibited a loss of one Chr18; the tumor with a 3-base pair deletion in PIEZO2 revealed a mosaic pattern concerning Chr18 status.

**Chr18-Related miRNAs Are Not Differentially Expressed**

Ten samples with and 10 samples without loss of Chr18 were analyzed concerning their Chr18-related miRNA expression profiles. In total, 27 miRNAs located on Chr18 were analyzed (see online suppl. table S7). Since no experimental data of expression evidence are available for miR-5011 and miR-5583, both mature sequences (3p and 5p) were analyzed. A total of 29 different miRNA assays were therefore excluded from further analysis. The statistical analysis of the remaining miRNAs that exhibited moderate to strong expression revealed no significant difference of expression between the 2 cohorts. Online supplementary table S8 shows significance values and fold changes of 15 miRNAs that showed high expression in the RT-PCR analysis. Only one miRNA was nearly significantly regulated (miR-1539; p = 0.06), and therefore the analysis of miR-1539 was extended to 40 samples in total (10 additional samples with and 10 without Chr18 loss). However, no increase in significance was detected (p = 0.26). The fold changes between the two cohorts ranged between −2.55 and 1.36. All results concerning the Chr18 associated tumor suppressors and miRNAs investigated in our study are summarized in online supplementary table S9.

**Discussion**

Most of SI-NETs are characterized by a loss of (a part) of Chr18 [11–13]. No other frequent genetic alterations or putative affected pathways have been implicated in the tumorigenesis of SI-NETs as yet. In our series of 138 SI-NETs, we found Chr18 loss in approximately 65% of the tumors. A number of studies (reviewed in [14]) investigated the possible effect of Chr18 loss on the genes that are located in this chromosomal region. As there are, however, no comprehensive data on Chr18-related alterations at the protein level in SI-NETs, we studied all 7 putative tumor suppressors (i.e. SMAD2, SMAD4, maspin, DCC, elongin A3, CABLES and PMAIP1) on Chr18 using RNA or protein-based assays, comparing SI-NETs with and without Chr18 losses.

SMAD2 and SMAD4 are important signal transduction molecules in the transforming growth factor-β (TGFβ) pathway [15] and are known to be functionally inactivated in different types of cancers. SMAD4 function is lost in pancreatic adenocarcinomas [16], metastatic colorectal cancer [17] and small intestine adenocarcinomas [18]. SMAD2 has also been described to be altered in a variety of cancers, although to a lesser extent than SMAD4 [19, 20].

In the SI-NETs of our series, SMAD2 and SMAD4 expression, tested by Western blot and IHC, remained unaltered, except for 3 tumors that showed loss of SMAD4 expression. These findings correlate with data from Löllgen et al. [21], who reported on preserved SMAD4 protein expression in 7 SI-NETs.

Maspin has shown to be downregulated in cancers of the breast, prostate and stomach, and in melanoma, but overexpressed in, e.g., colorectal, pancreatic and thyroid cancers. These contradictory findings may be explained by the special subcellular localization of the protein in cancer cells, which may be cytoplasmic or nuclear or both [22]. However, intensive studies have shed light on the tumor suppressor function of maspin by detecting reprogramming of the tumor proteome via maspin expression, particularly of protein pathways involved in tumor cell extravasation [23].

Maspin was not expressed in any of the 87 SI-NETs, but was present in the adjacent normal mucosa. This finding would make maspin an interesting candidate. However, the 100% loss of a protein during tumorigenesis is very unusual. We therefore examined maspin expression in the non-neoplastic single NE cells of the intestinal mucosa by immunofluorescent double staining for maspin and synaptophysin. This analysis revealed that maspin is only present in the mucin-producing cells of the normal ileal mucosa and absent in the NE cells. This suggests that the lacking maspin expression in neoplastic SI-NET cells does not reflect an oncogenic event but the phenotype of the non-neoplastic NE cells of the mucosa of the small intestine.

In 1990, DCC was identified to be frequently deleted in colorectal carcinoma [24]. Subsequently, reduced expression of DCC has been described in a variety of cancers [25, 26]. DCC encodes a netrin-1 receptor, which induces apoptosis in the absence of netrin-1 [27]. Abridged expression of DCC could result in less apoptosis and thus give rise to tumor progression. DCC is a 158.5-kDa transmembrane protein with at least 18 splice variants (7 being
were able to show that the TCEB3C gene was found to be silenced in ovarian, colorectal, and endometrial and non-small lung cancer. The specificity of the assay was verified with a biological positive control (IMR-32 cell line), competition of the reaction with a corresponding peptide and the mass spectrometry of the positive control. The mechanism of loss of DCC has yet to be unraveled. A possible explanation is alternative splicing. Remarkably, Francis et al. [3] reported the highest rate (29%) of intronic alterations in the DCC gene out of all putative tumor suppressor candidates on Chr18, rendering this gene even more interesting.

TCEB3C encodes for elongin A3, a transcription elongation factor identified in 2002 [9]. TCEB3C is a maternally imprinted gene on Chr18 [28], and hence only one mutational hit is required to fulfill the Knudson two-hit hypothesis. Edfeldt et al. [29] were able to show that TCEB3C is epigenetically repressed in the human SI-NET cell line CNDT2.5 due to histone and DNA methylation. Despite this, a high percentage of human SI-NETs in this study exhibited intact elongin A3 expression. We found that the qRT-PCR of TCEB3C in 69 FFPE samples revealed significant downregulation in SI-NETs with a loss of Chr18 compared to the samples with two copies (p = 0.0101). The comparison of the mRNA results with the protein expression was somehow limited, due to the fact that only one fresh frozen sample with normal Chr18 count was available for Western blot analysis. However, no loss of protein expression was detected in 21 fresh frozen samples. These results indicate that reduced mRNA expression was not associated with a relevant loss of protein.

CABLES is a cell cycle regulatory protein that plays a role in proliferation and differentiation [30]; the encoding gene was found to be silenced in ovarian, colorectal, endometrial and non-small lung cancer [31–34]. In 2013, the group led by Zukerberg reported that the (partial) loss of CABLES is also a frequent aberration in SI-NETs (Arason et al., 2013, Nature Abstract Laboratory Investigation). Our analysis exhibited a slight downregulation of CABLES (p = 0.0323) in SI-NET samples with Chr18 loss compared to tumors with diploid Chr18. However, the reduced mRNA expression was not associated with reduced protein expression; all samples showed strong protein expression in Western blot analysis. In a cohort of 25 SI-NETs, (partial) loss of CABLES was observed in 92% (n = 23) [35], but did not correlate with grade/stage/survival data. Whether the loss of CABLES expression was caused by the loss of Chr18 was not evaluated in this study.

PMAIP1 is a proapoptotic gene whose protein functions in a p53-dependent manner [36]. In 2008, PMAIP1 was identified as a potential tumor suppressor in pancreatic cancer by comparative cDNA microarray analysis [7]. In our study, the qRT-PCR of PMAIP1 revealed high Cp values, suggesting that PMAIP1-mRNA is low abundant in SI-NETs irrespective of their Chr18 status. The protein PMAIP1 was not expressed in any of our samples. Since only 8 cryo-samples were investigated, the explanatory power is somehow reduced. We think that PMAIP1, like maspin, is not present in NE cells at all. To confirm our hypothesis, we need to do a double immunofluorescence staining (synaptophysin + PMAIP1). However, no suitable antibody for PMAIP1 is available at the moment.

Our first systematic search for putative tumor suppressor proteins, which could get lost during tumorigenesis in SI-NETs, revealed only DCC as a promising candidate in one third of SI-NETs, although the mechanism of loss remains unclear. Our study has strengths and limitations. The strengths include the comprehensive approach to all Chr18-related events and the thorough examinations of the putative tumor suppressors at the protein level. The power of the study is limited by the low number of frozen tissue samples (14–21); however, most of the tumor suppressors exhibited an unequivocal expression profile, so that it was possible to draw clear conclusions. The second limitation concerns the lack of high-value antibodies for immunohistochemistry, making impossible to investigate high numbers of FFPE samples and to study co-localization of tumor suppressors in normal NE cells. The lacking loss of known tumor suppressors prompted us to proceed with additional genetic events on the remaining copy of Chr18 in order to identify novel putative tumor suppressor candidates that become inactivated by LOH. In this process, we analyzed 5 SI-NETs that showed complete (n = 4) or partial (n = 1) loss of Chr18 by exome sequencing. We found no SNVs or indels in known tumor suppressor genes in the 5 analyzed SI-NETs. To extend this search, we conducted data mining in a supplementary data set of the study by Banck et al. [4] and, again, were unable to find any relevant additional losses (SNVs in all 7 tumor suppressors investigated).

Regarding other Chr18-related genes, we found three somatic mutations by exome sequencing that could be validated by Sanger sequencing. One patient carried SNVs in the CABYR and NFATC1 genes, whereas a 3-bp deletion in PIEZO2 was present in another patient. The 3 remain-
Chr18-Related Alterations in SI-NETs

The authors did not mention if this line with recent data from Banck et al. validation experiments to more samples. These results, in online suppl. data 3), we decided against extending the Verdugo et al. Chr18-related alterations at the epigenetic level. We continued our comprehensive exploration of es of heterozygosity occur on Chr18, at least at the DNA level. We continued our comprehensive exploration of Chr18-related alterations at the epigenetic level.

Since the loss of one Chr18 may result in the down-regulation of Chr18-related miRNAs involved in tumor progression, we performed a comparative analysis of miRNA expression of 27 miRNAs in 10 samples with and 10 samples without loss of Chr18. In tumors, miRNAs can either function as oncogenes or tumor suppressors and have been shown to affect tumor progression of various kinds of cancer. In gastrointestinal stromal tumors, a partial loss of chromosome 14 went hand in hand with downregulation of Chr14-related miRNAs [10]. Two studies confirmed miRNA-133a, a Chr18-associated miRNA, to be downregulated during tumor progression [39, 40]. However, the authors did not mention if this downregulation is due to loss of Chr18. In our study, we found no significant downregulation of the 27 Chr18-related miRNAs in SI-NETs with loss of Chr18.

In summary, the question arises as to how one can explain the effect of the Chr18 loss in SI-NETs. One possible mechanism is haploinsufficiency of one of the putative tumor suppressors. Of the 7 putative tumor suppressor genes, it has been shown that SMAD4 haploinsufficiency significantly alters TGFβ and BMP (bone morphogenetic proteins) signaling, and that a SMAD4 dose-dependent transcriptional regulation of target genes of the TGFβ and Wnt signaling pathways in a SMAD4-mutant mouse model exists [41]. A second explanation may be provided by the study of Solimini et al. [42]. They were able to show that pro-proliferative genes (GO genes) are often enriched and anti-proliferative genes (STOP genes) are underrepresented by hemizygous deletions in malignant tumors. In other words, multiple haploinsufficiencies probably contribute to the proliferative fitness of cancers. This hypothesis may at least in part explain the significance of Chr18 loss in SI-NETs.

In conclusion, this first comprehensive study on alterations of putative tumor suppressor proteins on Chr18 in SI-NETs showed that DCC is the only tumor suppressor that is lost in 29% of cases, while SMAD2, SMAD4, CABLES and elongin A3 retained their expression. PMAIP1 and maspin were not expressed at all. This finding should be interpreted as a feature of NE cells in the small intestine in general rather than as a complete loss of these molecules during tumorigenesis. Exome sequencing of SI-NETs performed by us and others revealed no recurrent Chr18 alterations, which could lead to additional potential tumor suppressors. Regarding epigenetic regulation, there is no significant downregulation of all known Chr18-related miRNAs. The majority of epithelial tumors are triggered by driver genetic events, mostly by mutations. SI-NETs seem to be controlled by different mechanisms. Possibly, multiple monoallelic losses of tumor suppressor genes contribute to malignant transformation. If this is the case, further studies have to address this question using integrative analysis of genetic alterations and transcriptome and proteome signatures to shed light on the complex and subtle events in SI-NETs.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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